

# Effect of a Brief CO<sub>2</sub> Exposure on Ethylene Production

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## ABSTRACT

Ethylene production and respiration by Granny Smith apples were inhibited by treatment with 20% CO<sub>2</sub> for 2 hours. A similar effect was observed in tissue slices when treated at either 0 or 25°C.

The inhibition continued even after an extended aeration period. There is also an inhibition of ethylene emission in tissue slices incubated with exogenous 1-aminocyclopropane-1-carboxylic acid (ACC).

In general, CO<sub>2</sub> treatment increased the ACC content of the tissue. These observations are consistent with the idea the action of CO<sub>2</sub> is directed toward the enzyme system responsible for the conversion of ACC into ethylene.

The storage life of apples can be increased by treating the fruit with a high concentration of CO<sub>2</sub> during a short period.

Burg and Burg (9, 10) found that CO<sub>2</sub> is a competitive inhibitor of ethylene action, and Beyer (5, 6) reported that such an action is related with ethylene metabolism. CO<sub>2</sub> can affect this metabolism by inhibiting ethylene oxidation to CO<sub>2</sub>.

The action of CO<sub>2</sub> in delaying ripening rate has been related to a decrease in respiratory activity and an inhibition of the succinic oxidase complex and especially of the succinic dehydrogenase (18–20).

In addition to its respiration blocking action, CO<sub>2</sub> at high concentrations also decreases ethylene production in many fruits including apples, pears, tomatoes, etc. (7, 8, 14–16, 22). The pathway of ethylene production has been shown as methionine → SAM<sup>2</sup> → ACC → ethylene (2, 12).

This paper presents results on the effects of CO<sub>2</sub> at high concentrations on ethylene production and ACC levels in apple fruit tissue.

## MATERIALS AND METHODS

**Plant Material.** Granny Smith apples were harvested during the growing season of 1982 (April 1982). The fruits were stored in air at 0°C during a short period until used.

**Experiments with Whole Fruit.** A current of moist air or a mixture containing 10, 20, or 30% CO<sub>2</sub> was flushed (flow rate: 4 L h<sup>-1</sup>, for different periods of time, through jars containing one fruit.

**Experiments with Tissue Slices.** Discs (2 × 10 mm) of cortical tissue were incubated (about 1 g) in 25 ml containers with 3 ml of the following incubation medium: 0.6 M sorbitol, 10 mM K-

phosphate buffer (pH 6.8), and 50 µg/ml chloramphenicol (4).

In some experiments a HCO<sub>3</sub><sup>-</sup>-CO<sub>2</sub> buffer was used to control the CO<sub>2</sub> concentration of the gas phase (21). The experiments were carried out with 10, 20, and 30% of CO<sub>2</sub> at 0 and 25°C.

**Experiments with Exogenous ACC.** In some cases, ACC at 4 and 100 µM was added to the incubation media. The experiments were carried out at 0 and 25°C.

**Ethylene Determination.** Ethylene production from whole fruit was measured by GC using a flame ionization detector. Samples were taken at the exit of the flushing gas stream as well as in the inner part of the fruit. A glass tube closed at one end with a septum was inserted into the apple core through its open end for internal ethylene sampling.

**CO<sub>2</sub> Determination.** CO<sub>2</sub> was measured with a thermal conductivity detector GC fitted with a silica gel and molecular sieve columns.

**ACC Determination.** Once the experiment was over, the tissue slices were removed from the incubation solution, washed and ACC extracted with 80% ethanol. After evaporation, the residue was taken up into water and ACC determined by the method of Lizada and Yang (13).

**Statistical Analysis.** All experiments were repeated at least three times. The effects of CO<sub>2</sub> treatment were evaluated by analysis of variance and the least significant differences between means (LSD) at the 0.05 level were calculated.

## RESULTS

When whole fruits were treated with 20% CO<sub>2</sub> for either 2 h or 5 d, ethylene production was inhibited. An initial 2-h exposure to 20% CO<sub>2</sub> inhibited internal ethylene production by 28.4% after 5 d, whereas a continuous exposure to 20% CO<sub>2</sub> inhibited ethylene production by 80% after the same 5-d period.

Differences were larger in the inner part of the fruit than in the emitted gases making more evident an inhibition of ethylene production by high concentrations of CO<sub>2</sub>.

After CO<sub>2</sub> removal, ethylene production remained lower in the treated CO<sub>2</sub> than in the control fruit (Fig. 1), which showed the existence of a residual effect. This effect was more evident in fruit treated with high CO<sub>2</sub> during 5 d.

The inhibition of ethylene production was higher as the CO<sub>2</sub> concentration was increased (Table I).

Respiration was also decreased by high CO<sub>2</sub> treatment (Table I), a residual effect being observed after the removal of the latter.

**Effect of CO<sub>2</sub> on C<sub>2</sub>H<sub>4</sub> Production in Tissue Slices.** The inhibitory effect of CO<sub>2</sub> on ethylene production in apple discs is shown in Figure 2. As in whole fruit, the degree of inhibition increased (Fig. 1) as the CO<sub>2</sub> concentration and exposure period were increased. The CO<sub>2</sub> effect was more evident at 25°C than at 0°C.

By eliminating CO<sub>2</sub> from the medium, either by airing the containers or replacing the medium with an aired incubation medium, it was found that at the end of an incubation period (which was twice the duration of the CO<sub>2</sub> treatment) the reduc-

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<sup>2</sup> Abbreviations: SAM, S-adenosyl-L-methionine; ACC, 1-aminocyclopropane-1-carboxylic.

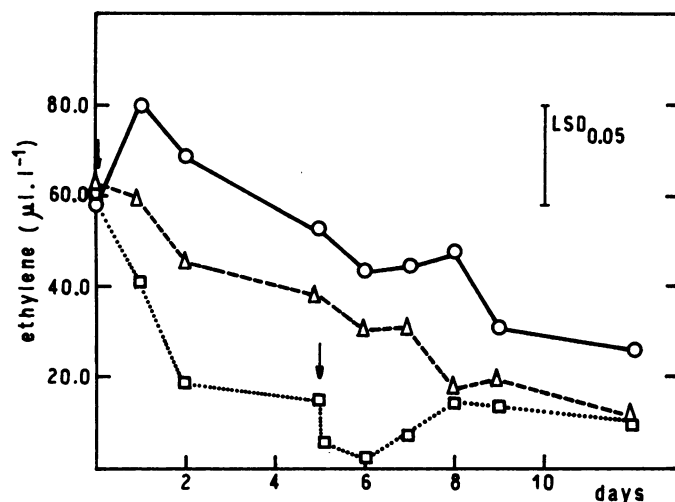


FIG. 1. Internal ethylene production in Granny Smith apples treated with 20% CO<sub>2</sub> during 2 h (Δ---Δ) and 5 d (□...□) compared with the ones kept in air (O—O) (the arrows show the end of the treatment). Temperature: 20°C.

Table 1. Ethylene and Respiration Inhibition in Granny Smith Apples with Different Concentrations of CO<sub>2</sub> during 2 Hours and 3 Days at 20°C

CO <sub>2</sub>	Ethylene Inhibition		Respiration Inhibition	
	2 h	3 d	2 h	3 d
%	%	%	%	%
0.03	0.0	0.0	0.0	0.0
10	3.1	31.9		27.2
20	10.8	64.1	11.6	62.6
30	24.3	85.0	17.4	73.0

tion in ethylene production in the CO<sub>2</sub> treated discs still continued (Table II). This result confirmed the residual effect observed in the whole fruit (Fig. 1). The same residual inhibition of respiration was observed (Table II).

**CO<sub>2</sub> Effect on Ethylene Production from Exogenous ACC.** When ACC was added to the incubation medium at 0.10 mM, a stimulation of ethylene production was observed which was approximately 60 times that of the controls. This ethylene production was also inhibited by the presence of CO<sub>2</sub> (Table III).

The same result was obtained at 0 and 25°C and at both ACC concentrations (4 mM and 100 μM).

**CO<sub>2</sub> Effect on the Endogenous ACC Content.** The ACC content of tissue slices in air decreased rapidly during the first 6 h of incubation either at 0 or 25°C. For longer periods of time at 0°C it continued to decrease but more slowly. In contrast, at 25°C the ACC content started to increase between the 6th and 10th hour (Table IV), probably due to tissue senescence.

The ACC content in tissue slices did not vary with different temperatures for incubation periods of less than 6 h (Table IV).

Adding 20% CO<sub>2</sub> to discs incubated at 0°C resulted in a slower decline of ACC and this decline started at about 6 h after incubation (Table IV).

At 25°C, with 4 h incubation and in the presence of CO<sub>2</sub>, there was an increase in endogenous ACC content with respect to the controls. By the end of 10 h incubation the ACC level started to increase, as in the controls, that level being lower in the CO<sub>2</sub>-treated discs (Table IV). This diminution was greater when the percentage of CO<sub>2</sub> in the atmosphere increased.

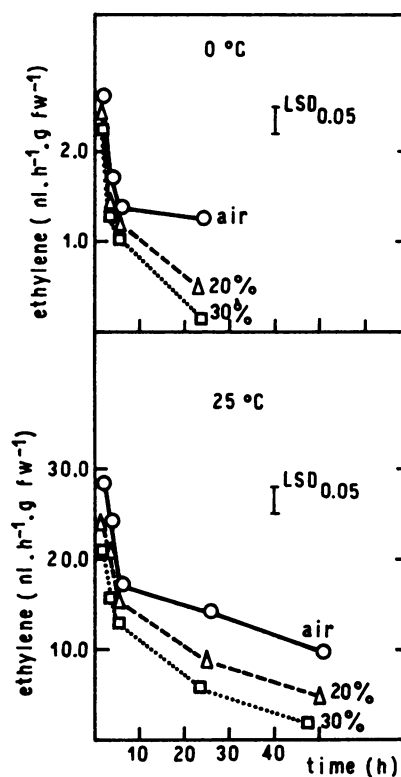


FIG. 2. Ethylene production of cortex tissue slices of Granny Smith apples incubated in air, 20 and 30% CO<sub>2</sub> during the indicated periods at 0 and 25°C.

Table II. Ethylene and CO<sub>2</sub> Production of Apple Cortex Slices

The slices were incubated in air or in 20% CO<sub>2</sub> during 2 or 24 h and then in air 4 or 48 h at 25°C.

Time	Ethylene Production		Respiration Rate	
	Air	20% CO <sub>2</sub>	Air	20% CO <sub>2</sub>
h	nl/h·g fresh wt		μl CO <sub>2</sub> /h·g fresh wt	
2 + 4	9.34	3.23	31.35	27.02
24 + 48	4.32	2.79	24.76	20.30
LSD <sub>0.05</sub>	0.62		0.97	

After airing the discs, the ACC level was slightly higher in the ones incubated in CO<sub>2</sub> at both temperatures (Table V).

Whole fruit were treated during 3 d with 20% CO<sub>2</sub> at 0°C for determination of the ACC content; after being analyzed it was found that the ACC content was higher in the treated fruit (4.83 versus 5.92 nmol ACC/g in controls and treated fruit, respectively). This situation remained the same after 7 d of aeration at 25°C (2.01 and 4.72 nmol ACC/g, respectively). In both the controls and treated fruit stored at 25°C, the ACC content increased when the treatment period increased and the difference between them tended to disappear (for 30 d at 25°C: 91.86 versus 98.76 nmol ACC/g, respectively).

## DISCUSSION

As reported, CO<sub>2</sub> at high concentrations of 10 to 30% inhibited ethylene production in apple cortex slices and in whole fruit (Figs. 1 and 2). Likewise, respiration is decreased due to an alteration of mitochondrial activity (20).

In 1981, Apelbaum *et al.* (4) reported that apple discs convert

Table III. Effect of ACC on Ethylene Production of Granny Smith Apple Discs

The discs were incubated either in absence or presence of 4  $\mu\text{M}$  and 100  $\mu\text{M}$  ACC in air or in 20%  $\text{CO}_2$  during the indicated periods at 25°C.

Time	Ethylene Production		Inhibition
	Air	$\text{CO}_2$	
<i>h</i>	<i>nl/h·g</i>		%
Control			
6	8.40	7.65	8.9
24	6.95	3.33	52.1
LSD <sub>0.05</sub>	0.81		
ACC 4 $\mu\text{M}$			
6	11.90	10.72	9.9
24	9.18	4.27	53.5
LSD <sub>0.05</sub>	0.60		
ACC 100 $\mu\text{M}$			
6	982.96	340.26	65.38
24	555.00	248.06	55.30
LSD <sub>0.05</sub>	64.22		

Table IV. Endogenous ACC Content of Granny Smith Apple Discs

The discs were incubated in air or in the presence of 20%  $\text{CO}_2$  during the indicated periods of time at 0 and 25°C.

Time	ACC Content in	
	Air	$\text{CO}_2$
<i>h</i>	<i>nmol/g</i>	
Temperature 0°C		
0	4.00	4.00
2	1.11	1.08
4	0.82	0.75
6	0.45	0.67
10	0.40	0.65
24	0.27	0.57
Temperature 25°C		
0	4.00	4.00
2	2.03	1.84
4	0.62	1.11
6	0.39	0.66
10	0.75	0.77
24	2.30	1.46
48	5.66	2.83
LSD <sub>0.05</sub> between treatments		0.27

Table V. Endogenous ACC Content of Granny Smith Apple Discs

The discs were incubated in air during the indicated periods of time or in 20%  $\text{CO}_2$  during 2 or 24 h and then in air during 4 or 48 h at 25°C.

Time	ACC Content in	
	Air	$\text{CO}_2$
<i>h</i>	<i>nmol/g</i>	
2 + 4	0.84	0.98
24 + 48	0.88	1.07
LSD <sub>0.05</sub>	0.13	

exogenous ACC to ethylene through the natural physiological system. In order to establish how  $\text{CO}_2$  acts, we studied its effect on the conversion of ACC to ethylene in tissue slices. A 20%  $\text{CO}_2$  treatment inhibited this conversion indicating that  $\text{CO}_2$  influences  $\text{C}_2\text{H}_4$  biosynthesis by affecting the last step in the ethylene biosynthesis sequence.

Adams and Yang (2) reported ACC to be the immediate precursor of  $\text{C}_2\text{H}_4$ . The oxidation of the latter would produce

$\text{C}_2\text{H}_4$  as well as  $\text{CO}_2$  (1). Thus,  $\text{CO}_2$  could act through a mass action effect in the inhibition of this reaction. However, when discs were treated with  $\text{CO}_2$  and then aired, they continued to produce less ethylene. This clearly indicates that the  $\text{CO}_2$  effect is not readily reversible. Therefore, the inhibition of ethylene production could not be due to a simple mass effect.

In the same way, a higher level of endogenous ACC was found in apple discs incubated for more than 4 h at 0°C and during 4 and 6 h at 25°C. For longer incubation periods at this temperature we must take into account the tissue senescence and/or the tissue alteration effect on the conversion of ACC, which is strongly diminished. Mayak *et al.* (17) reported that the conversion of ACC to ethylene becomes rate limiting with tissue senescence, resulting in an accumulation of ACC, as it is shown in Table IV. When delaying senescence,  $\text{CO}_2$  probably produces a lower accumulation of ACC, and even a lower one when the percentage of  $\text{CO}_2$  is higher.

Since the enzyme or enzymes involved in the conversion of ACC to ethylene is or are linked to membranes (3, 4, 11), the  $\text{CO}_2$  action could be due to an effect on them. The higher content of ACC found in discs treated with  $\text{CO}_2$  and then aired confirms the partial reversibility of the effect of  $\text{CO}_2$ . The results obtained with whole fruit were the same as the ones found for tissue slices.

To be able to elucidate the action of  $\text{CO}_2$  at high concentrations it will be necessary to study the enzyme or enzymes involved in ethylene biosynthesis.

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